**Reviewer’s report**

**Title:** Musculoskeletal application and validation of speckle-tracking ultrasonography

**Version:** 0  **Date:** 10 Dec 2018

**Reviewer:** John Drazan

**Reviewer's report:**

Review:

Notes to author:

The purpose of this study was to develop a new modality for characterizing muscle function using ultrasound speckle tracking in the biceps and supraspinatus. The test cohort was a convenience sample of 10 subjects with no history of neuromuscular disorder. Muscle deformation was characterized using ultrasound speckle tracking while subjects performed cyclic isometric contractions against a custom dynamometer at several magnitudes normalized relative to a subject's maximal voluntary contraction. The correlation between muscle strain and force generated was tested using a one-way ANOVA. Results showed that observed strain characterized using ultrasound speckle tracking was correlated with contraction magnitude.

This study is interesting because it demonstrates the feasibility of characterizing muscle function using clinically available equipment, however there are extensive issues with word choice throughout the paper (e.g. incorrect definition of strain and changing between the word cycles in line 148 and loops in 167 among others) that make the paper difficult to understand. Additionally, there are sections of the methods that are not reported in the results, for example retesting five days later.

**Introduction:**

The introduction is OK, however there are several issues regarding word choice and framing the study. If the SEMG is the gold standard for evaluating muscular function, why wasn't SEMG used as the comparison rather than isometric dynamometry? Why would isometric dynamometry be used as a comparison at all? The value of isometric dynamometry is not discussed until the discussion section.

Also, sarcomeres are too small to be evaluated using ultrasound. Strain, deformation, and tension seem to be used interchangeably and this is confusing. What is actually important and what is being measured? Ultrasound speckle tracking has already been used for skeletal muscle as early as 1989. Therefore, the hypothesis of "ultrasonic speckle patterns can be tracked in vivo
in skeletal muscle" has already been demonstrated. Instead it seems like you hypothesized and tested that strain measure using ultrasonic speckle tracking is correlated with submaximal isometric contractions.

Methods:

The methods are well described; however, they are confusing. A figure or picture of the custom dynamometer would be helpful to visualize how this testing was performed. Is STU a real time process where it takes place while each ultrasound frame is captured? Or is the ultrasound data captured, and later analyzed? It is unclear to me whether the described "STU" refers to the speckle tracking analysis or the collection of data. For data analysis, what is Q-analysis? Is this a software package? Also, the methods switched from cycles to loops at line 166. If these are different things, I have no idea what a loop is. Overall, what values are established a priori to indicate an accurate test?

Results:

You mentioned doing test-retest repeatability. Where did this go?

Discussion:

The discussion seems to draw conclusions not supported by the data collected. How can you say, "high accuracy?" How does this approach compare to previous studies using SEMG or other modalities? In line 201, you use the word invented. I suggest "proposed" instead because ultrasound speckle tracking has been used for decades. Overall, the words deformation, strain, and tension are used seemingly interchangeably. Each of these terms have specific, technical definitions which while have similarities, are unique. How are STU measurements independent of probe pressure?

General notes:

* The purpose of this study was to analyze muscle contractile properties using ultrasound speckle tracking.

* Is electromyography the gold stand for assessment of muscle performance? Line 65

* Sarcomere tension vs fascicle tension. Line 65
* Tension vs contractility line 74


* Awkward sentence at 105. What is happening here?

* GE Software? Line 143

* Cine loop? Line 144

* Why wasn't STU performed during the MVC 119?

* What is Q-analysis? Line 164

* What are the properties of the custom made dynamometer?

* What is a cycle, and what is a loop? Line 166

* For results, what about the retest protocol mentioned in line 161

* Line 210, if isometric dynamometry is bad, why are you using it to validate your system.

* Line 201: Speckle tracking using ultrasound is a well documented technique within the field of biomechanics as early as 1989. The word invention is most likely not appropriate to describe the work done here.

* If EMG is presented as the gold standard, why isn't that the comparison?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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